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by synchrotron infrared microspectrometry**

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# Chemical imaging of living cells by synchrotron infrared microspectrometry.

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## ABSTRACT

Chemical mapping of proteins and lipids inside a single living cell and at a resolution of a few microns, has been performed using synchrotron infrared microspectrometry. Modifications of the chemical distributions upon mitosis and necrosis has been investigated.

**Keywords:** living cell, proteins, lipids, infrared, synchrotron, microspectrometry

Individual cells are quite small (10 to 30  $\mu\text{m}$  in diameter) and their composition is complex. Their molecular composition is hardly determined and it is difficult to understand the function of the various components. What we can learn about cells depends on the probe technique. Numerous light microscopic techniques exist. Standard light microscopy (resolution up to 0.2  $\mu\text{m}$ ) utilizes fixed and stained cells while immunofluorescence microscopy reveals specific proteins and organelles. Transmission electron microscopy visualizes detailed structure of membrane and organelles (resolution up to 2 nm) and scanning electron microscopy reveals the details on the outer shell of cells and particles. In this paper we demonstrate that molecular compound distributions (proteins, lipids and nucleic acids) are obtained with a spatial resolution of few microns inside cells without the use of stains and fixatives using synchrotron infrared microspectrometry.

Infrared spectroscopy is a well-established, non-destructive analytical tool which is extensively applied to identify chemical groups in compounds from different sources (physics, chemistry and biology) <sup>1</sup>. The identification of the chemical groups rely on the dependence of the positions, widths and strengths of the vibration modes of the molecule upon the chemical structure. The identification of the vibrational modes of the major compounds (nucleic acids, proteins, lipids) of biological tissues and cells has been performed using conventional Fourier transform infrared spectroscopy<sup>2-4</sup>. Thus infrared spectroscopy provides a mean to image chemical compounds inside the cells. The major challenge is to attain the appropriate spatial resolution as with conventional infrared microspectrometry only images of area greater than 100  $\mu\text{m}^2$  are obtained with a sufficiently good signal-to-noise ratio. It has recently been demonstrated that using the high brightness of the synchrotron source combined with a commercial doubly confocal microscope, resolution up to few microns are achieved<sup>5, 6</sup>. We demonstrate hereafter, that detailed chemical images (in micron scale range) of individual cells are obtained using synchrotron infrared microspectrometry.

## MATERIALS AND METHODS

UN2.C3 cells were derived from the mouse UN2 hybridoma B cells<sup>7</sup>. They were cultured in RPMI 1640 medium supplemented with 10% FCS, penicillin (50 IU/ml) and streptomycin (50 mg/ml). To record IR spectra, the cells were deposited on a transparent IR window (Barium fluoride, BaF<sub>2</sub>) by low speed cytopspin centrifugation. Their viability was tested before and after recording the spectra using trypan blue method.

IR spectra are recorded using a Spectra Tech Ir $\mu$ s<sup>TM</sup> scanning infrared microspectrometer and the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory. Spectra were recorded in transmission with an instrumental resolution of 4  $\text{cm}^{-1}$  and 128 scans were co-added prior to Fourier transform processing. Each spectrum is ratioed to a reference one, recorded with the same parameters and conditions but outside the cell. After Fourier transform the spectra were processed according to the following procedure:

- Linear baseline has been first subtracted , in order to account for the continuous decay of the beam intensity inside the storage ring.
- Integrated intensity of the band or massif under consideration has been calculated. .

## RESULTS

### *Spectrum of a single cell*

The first step toward mapping chemical functions inside a cell lies in the ability to record IR spectra of high signal-to-noise ratio within an area much smaller than the dimensions of a cell. Such a possibility is demonstrated here on figure 1 which displays a spectrum acquired with a transmitted beam collected through an aperture of  $3 \times 3 \text{ micron}^2$  (about 1/100th of cell area). High signal-to-noise ratio spectrum is obtained in only 55s. This signal-to-noise ratio is severely reduced below  $1200 \text{ cm}^{-1}$  due to the combination of the diffraction effects ( diffraction starts for a wavelength egal to the aperture size ) and the change of the reflective index of the material used as the substrate ( in the present case :  $\text{BaF}_2$  ) . A compromise should be found between the desired resolution and the spectral region , because when using double confocal objectives, the lateral resolution will be kept the same for all the frequencies but at the expense of intensity loss for long wavelength. In addition, if there is any change of the reflective index of the substrate, the two apertures in the confocal configuration are not anylonger conjugated, resulting in a drastic loss of intensity at the detector (see figure 2 and discussion). In the present work, using an aperture size of  $3 \times 3 \text{ micron}^2$  and a  $\text{BaF}_2$  substrate, spectrum of high signal-to-noise ratio could be obtained up to about  $1200 \text{ cm}^{-1}$  ( $8.5 \text{ }\mu\text{m}$ ).

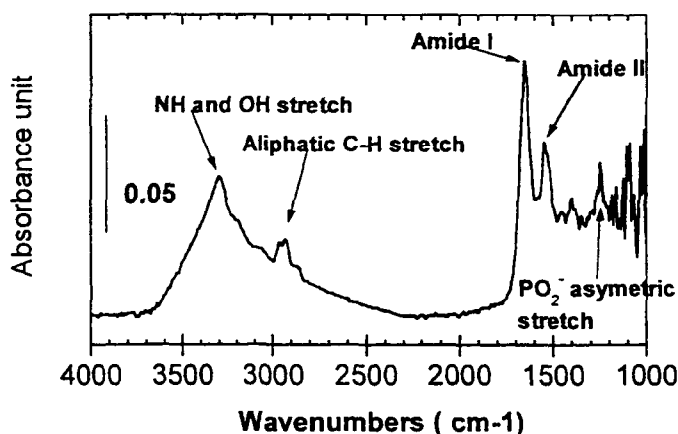


Figure 1 : Infrared spectrum of a mouse UN2 hybridoma B living cell, recorded with an aperture size of  $3 \times 3 \text{ }\mu\text{m}^2$  (See Materials and methods).

The high complexity of the cell chemical composition results in the superposition of the absorption bands of the differents components of the cell and thus few large absorption bands are observed. Identification of each component is precluded but the main chemical classes (proteins, nucleic acids, lipids ) can be identified through the assignment of one or more of the vibrational band of their characteristic functional groups. Assignments of these vibrational bands have been the subject of previous work<sup>8-10</sup>.

The band at  $1240 \text{ cm}^{-1}$  originates from the asymmetric stretching mode of phosphodiester groups ( $\text{PO}_2^-$ ) in nucleic acids.

The bands at  $1545 \text{ cm}^{-1}$  (amide II) and  $1650 \text{ cm}^{-1}$  (amide I) arise fom vibrations of amide groups of proteins. The  $\text{H}_2\text{O}$  deformation band can also contribute to the intensity of the amide I band at  $1650 \text{ cm}^{-1}$ .

The bands in the  $2850\text{--}2960\text{ cm}^{-1}$  region arise from symmetric and asymmetric stretching modes of methylene ( $\text{CH}_2$ ) and methyl ( $\text{CH}_3$ ) groups found in proteins and lipids.

The band at  $3300\text{ cm}^{-1}$  originates from the vibrations of OH and NH groups ( $\text{H}_2\text{O}$ , proteins and polysaccharides).

Thus it is possible to map unambiguously nucleic acids and proteins in cells by the analysis of the band at  $1240\text{ cm}^{-1}$  and the amide II band at  $1545\text{ cm}^{-1}$ , respectively. Mapping the lipids will require that the  $\text{CH}_2$  and  $\text{CH}_3$  functional groups of proteins do not contribute significantly to the bands in the  $2850\text{--}2960\text{ cm}^{-1}$  spectral region.

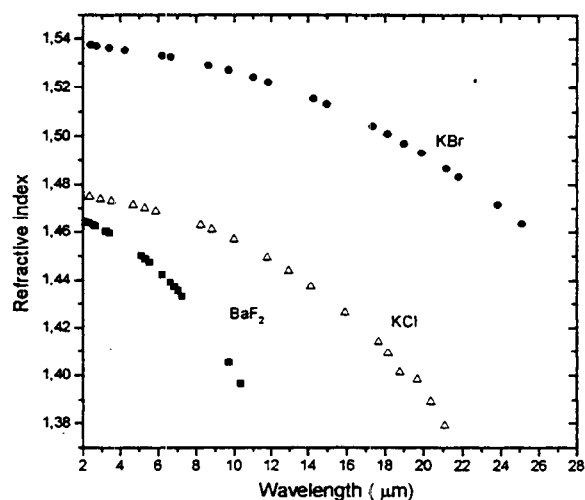


Figure 2. Curve of the refractive index versus the wavelength<sup>11</sup>.

#### Linear mapping

The next step toward chemical mapping is the ability to distinguish two close areas of the sample. To demonstrate this, we acquired a series of spectra through an aperture of  $3 \times 3\text{ micron}^2$ , with a step of  $1\text{ }\mu\text{m}$  along an axis crossing a cluster of four cells, as pictured on figure 3. The graphs on figure 4 display the value of the integrated intensity for each band versus the position of the beam. The concentration profile for each cell and inside each cell is well defined.

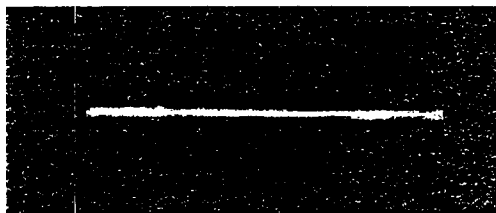
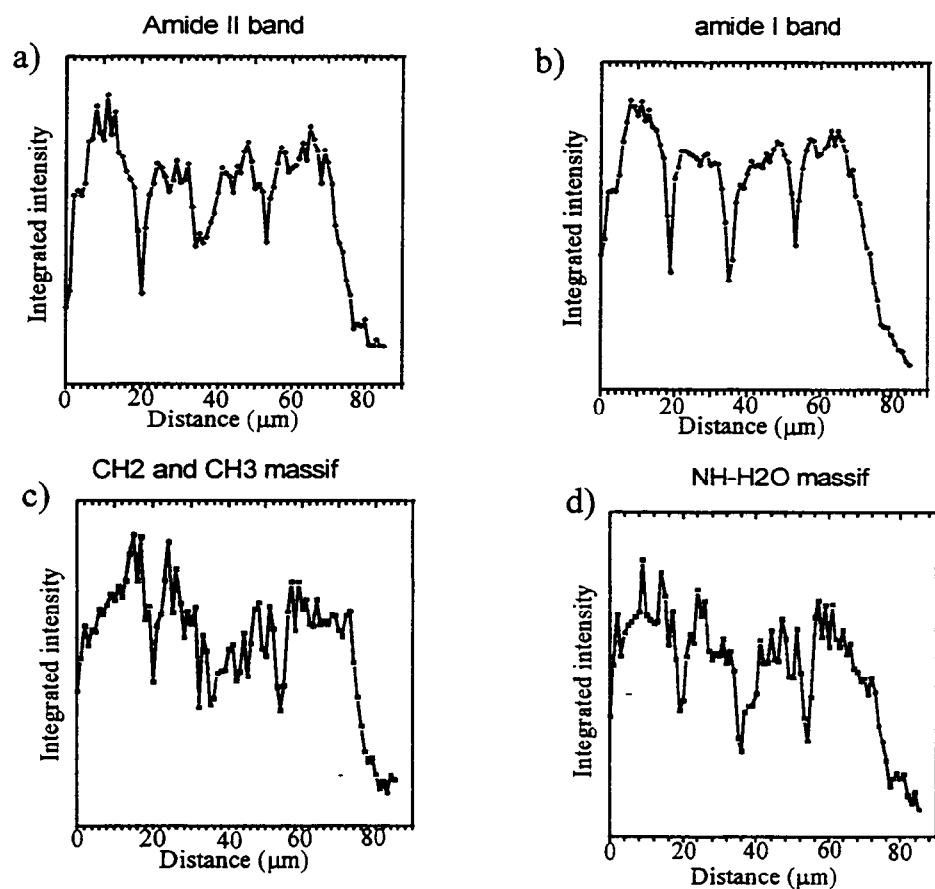


Figure 3: Optical image of the cells cluster mapped linearly

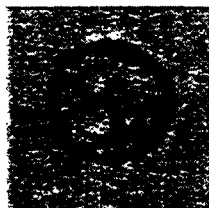


*Figure 4.* Linear mapping of a cluster of cells. Infrared spectra were recorded every 1  $\mu\text{m}$  along an axis crossing the four cells. a , b, c, d: Plots of the integrated intensity of respectively Amide II band, Amide I band,  $\text{CH}_2$  and  $\text{CH}_3$  massif, NH and OH massif, versus position of the beam.

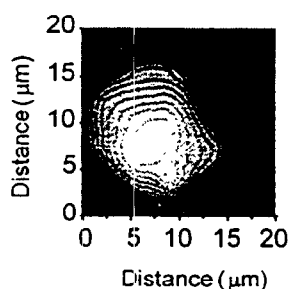
#### *Chemical mapping of cells.*

We then acquired, in a raster fashion, a matrix of  $14 \times 14$  spectra through an aperture of  $3 \times 3 \text{ micron}^2$  stepping every 3  $\mu\text{m}$  of a single living cell. The distribution of the integrated intensity of the amide II band and the  $\text{CH}_2$  asymmetric stretching band ( $2929 \text{ cm}^{-1}$ ) of the cell is reported on figure 5. The amide II band image is quite homogeneous and the intensity is maximum around the center of the cell. This is in agreement with the large size of the nucleus of this type of cell. In contrast, the distribution of the  $\text{CH}_2$  groups is non homogeneous and different than those of proteins which implies that it represents mainly the distribution of lipids inside the cells. This observation was made for all the different cells investigated.

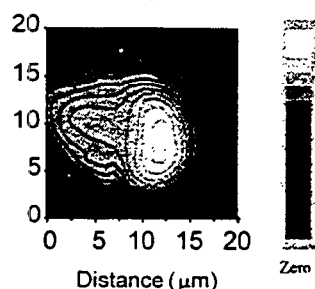
### A. Optical Image



### B. Proteins



### C. Lipids

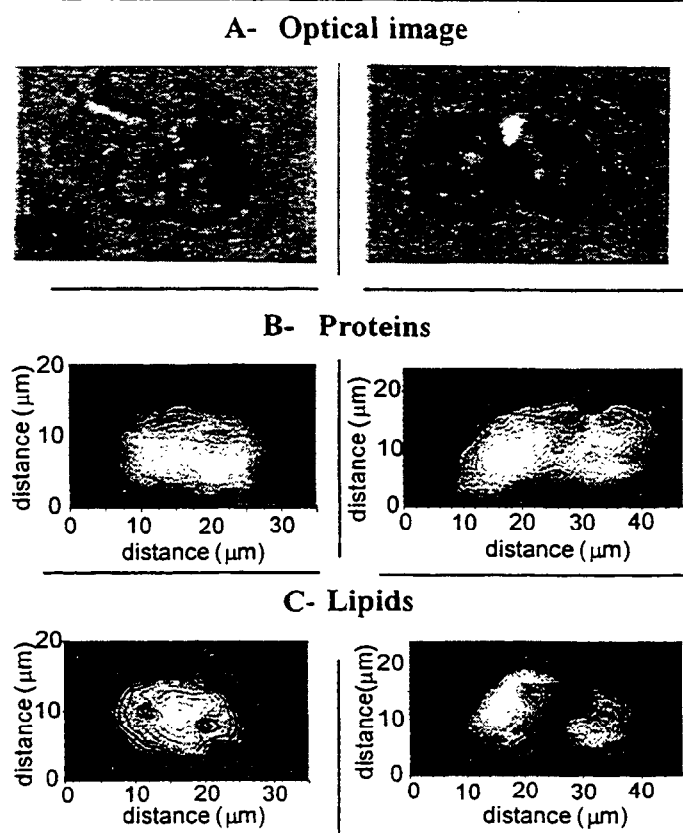


*Figure 5.* Chemical distribution of the proteins and lipids characteristics IR bands ( amide II and CH<sub>2</sub> asymmetric stretching bands respectively). A: Optical image. B: Protein distribution. C: Lipid distribution. The mappings have been obtained by analysing the integrated intensity variation of a two-dimensional set of infrared spectra (14x14 points, step equal to 3  $\mu\text{m}$ ). Each spectrum was recorded in transmission through a 3X3  $\mu\text{m}^2$  aperture.

The feasibility of mapping chemical classes of compounds open the way to study variations in the distribution of these compounds during biological events.

We studied proteins and lipids distributions in cells at late stages of mitosis. Figure 6a displays the optical image of a cell at the late telophase stage with an almost complete cytokinesis (left panel) and the optical image of cells which likely derive from a complete mitosis (right panel). For the cell at the late telophase stage, the protein distribution is spread out all over the cell but with maxima centered in the two regions which correspond to the daughter cells (Figure 6b, left panel). The lipids distribution is particularly high in the region where the contractile ring responsible for the cleavage furrow is located (Figure 6c, left panel). This could correspond to the inward pulling of the lipids by this contractile ring occurring in that region just before cell division. In contrast, when the cells are separated, the lipids and proteins distributions are similar to those observed in isolated cells (see Figures 6c and 5).

We have also studied the proteins and lipids distributions for cells undergoing necrosis. The IR spectrum of such a cell (Figure 7) shows broader bands than in the case of viable resulting from an increased heterogeneity of the sample. A new narrow band appears at 1730  $\text{cm}^{-1}$  and is assigned to carbonyl ester groups, different from carbonyl groups of phospholipids which appears as a shoulder at 1740  $\text{cm}^{-1}$ . The carbonyl ester distribution departs from the proteins and lipids distribution as shown in Figure 7.



*Figure 6.* Chemical distribution of proteins and lipids of cells undergoing mitosis. A: Optical image. B, C : Proteins and lipids distribution, respectively. The mappings result from the analysis of sets of infrared spectra: left panels (B and C), 18x11 spectra every 2  $\mu\text{m}$  (aperture 5x5  $\mu\text{m}^2$ ) and right panels (B and C), 13x9 spectra every 3  $\mu\text{m}$  (aperture 3x3  $\mu\text{m}^2$ ).

## CONCLUSION

High quality IR spectra and chemical mappings of cells are obtained when using the high brightness synchrotron infrared radiation source at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory, in combination with a Spectra Tech Ir $\mu\text{s}$ <sup>TM</sup> scanning microspectrometer.

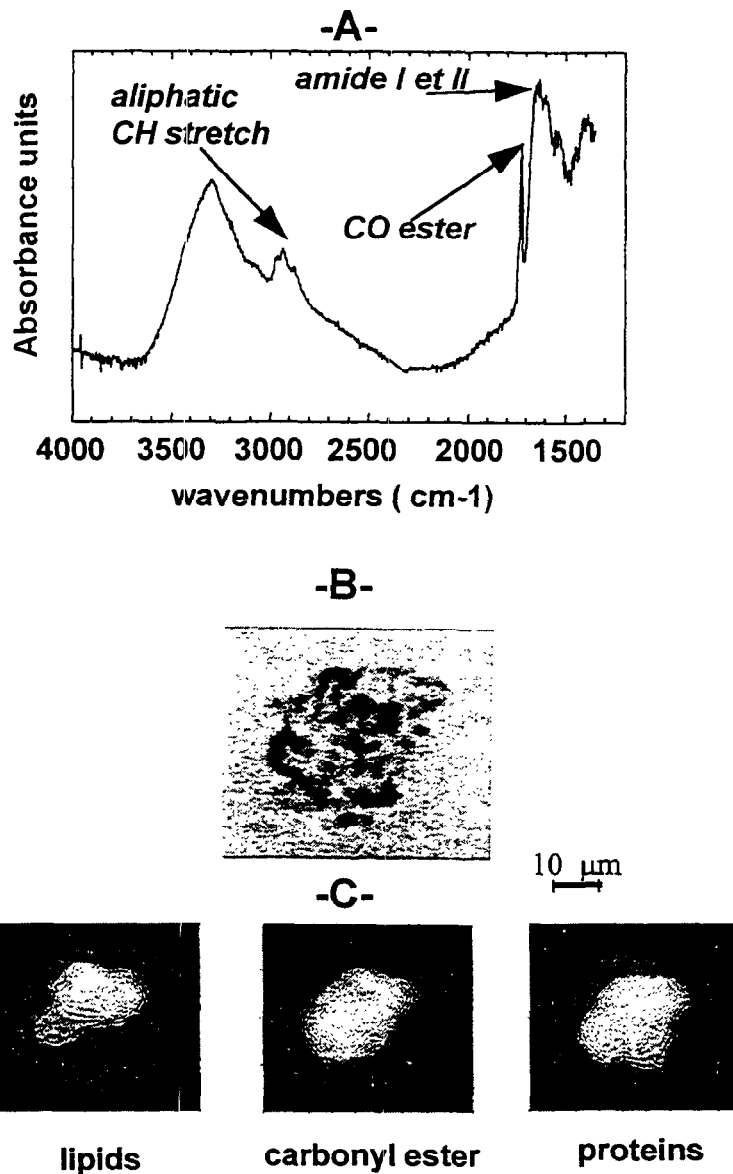
Reduction of the acquisition time is possible as the instrumental resolution could be degraded by , at least a factor of four, due to the broad lineshape of the IR bands (30  $\text{cm}^{-1}$ ). Moreover, the number of co-added scans could also be diminished by a factor of four or more, without decreasing too much the signal-to-noise ratio. Consequently the recording time of the chemical images display on figure 3 can be reduced by a factor of 16.

The quality of the spectra in the region below 1300  $\text{cm}^{-1}$  should be greatly improved by the use of other substrate like , for example, KBr window , which are commercially available. As shown on Figure 2, the decrease of the refractive index of KBr is less important in the region 2.5 to 20  $\mu\text{m}$  than for BaF<sub>2</sub>. The infrared spectrum in the region 1300-500  $\text{cm}^{-1}$  is composed of numerous bands. Some of them have been assigned e.g. the asymmetric  $\text{PO}_2^-$  stretching band, the C-O stretching mode of the C-OH groups of serine, threonine, and tyrosine in proteins as well as C-O groups of carbohydrates. With the use of the KBr substrate mapping nucleic acids and C-OH groups will be feasible and will bring relevant



biochemical information. Using a CuGe detector, instead of a MCT one, will also contribute to improve the signal-to-noise ratio in the 1000-600  $\text{cm}^{-1}$  spectral region.

Applications of synchrotron infrared microscopy in the field of cellular biology are very promising, for example in comparing different types of cells, and their modification upon drug treatment or radiations. One particular aspect would be the time resolved aspect of such a technique, which should deserve a particular interest in a near future.



**Fig7 :** Infrared spectrum and chemical distributions of functional groups of a necrotic cell. A: Infrared spectrum recorded with an aperture of  $5 \times 5 \mu\text{m}^2$ . The instrumental resolution was set at  $4 \text{ cm}^{-1}$ , and the spectrum displayed is the result of 128 coadded scans. B: Optical image. C: Lipid, carbonyl ester group, and protein distributions. The chemical distributions are the result of the analysis of  $15 \times 14$  spectra, each spectrum is recorded every  $3 \mu\text{m}$  step using an aperture size of  $5 \times 5 \mu\text{m}^2$  and a  $4 \text{ cm}^{-1}$  resolution (128 scans per spectrum).

## REFERENCES

1. P.R.Griffiths, J.A. de Haseth, "Fourier Transform Infrared Spectroscopy", John Wiley and Sons Eds., New York, 1986.
2. P.T.T. Wong and B. Rigas, Appl. Spectrosc. 44, 1715, 1990.
3. P.T.T. Wong and B. Rigas, Appl. Spectrosc. 4, 1563 (1991).
4. P.T.T. Wong, R.K. Wong, T.A. Caputo, T.A. Godwin, and B. Rigas, Proc. Natl. Acad. Sci. (USA) 88, 10988, 1991.
5. G.L. Carr, J.A. Reffner, G.P. Williams, Rev. Sci. Instrum. 66, 1490, 1995.
6. G.L. Carr, M. Hanfland, G.P. Williams, Rev. Sci. Instrum. 66, 1643, 1995.
7. S. Brunati, C. Miossec, C. Mathiot, J. Moncuit, S. Amigorena, J.-L. Teillaud, W.H. Fridman, Mol. Immunol., 25, 1133, 1988.
8. P.T.T. Wong, E.D. Papavassiliuo, B. Rigas, Appl. Spectrosc. 45, 1563, 1991.
9. H.Susi and D.M. Byler, Methods Enzymol. 130, 290, 1986.
10. F. Siebert, Methods Enzymol. 246, 501, 1995; W.L. Peticolas, ibid. 246, 389, 1995.
11. The Infrared Handbook Office of Naval Research Department of the Navy , Editors: Wolfe and Zissis , 1985.